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ORIGINAL PAPER

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Structures of coenzyme F_{420} in *Mycobacterium* species

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Abstract The structure of coenzyme F_{420} in *Mycobacterium smegmatis* was examined using proton NMR, amino acid analysis, and HPLC. The two major F_{420} structures were shown to be composed of a chromophore identical to that of F_{420} from *Methanobacterium thermoautotrophicum*, with a side chain of a ribityl residue, a lactyl residue and five or six glutamate groups (F_{420}–5 and F_{420}–6). Peptidase treatment studies suggested that L-glutamate groups are linked by γ-glutamyl bonds in the side chain. HPLC analysis indicated that *Mycobacterium tuberculosis*, *Mycobacterium bovis* BCG, and *Mycobacterium fortuitum* have F_{420}–5 and F_{420}–6 as the predominant structures, whereas *Mycobacterium avium* contains F_{420}–5, F_{420}–6 and F_{420}–7 in significant amounts. 7,8-Didemethyl 8-hydroxy 5-deazariboflavin (FO), an intermediate in F_{420} biosynthesis, accounted for about 1–7% of the total deazaflavin in cells. Peptidase treatment of F_{420} created F_{420} derivatives that may be useful for the assay of enzymes involved in F_{420} biosynthesis.

Keywords Coenzyme F_{420} · FO · *Mycobacterium* · Polyglutamate · 5-deazaflavin · Folate

Introduction

F_{420} is a two-electron transfer coenzyme that is present in some members of three distantly related groups of prokaryotes: the archaea, the aerobic actinomycetes, and the cyanobacteria. Very few other genera contain F_{420}. In archaea, F_{420} is essential for hydrogenase, formate dehydrogenase, methylene-tetrahydromethanopterin dehydrogenase, alcohol dehydrogenase, methylene-tetrahydromethanopterin reductase, and quinone oxidoreductase reactions (Jones and Stadtman 1980; Jacobson et al. 1982; Hartzell etal. 1985; Widdel and Wolfe 1989; DiMarco et al. 1990; Ma and Thauer 1990; Daniels 1994; Kunow et al. 1994). F_{420} and/or 7,8-didemethyl 8-hydroxy 5-deazariboflavin (FO, a biosynthetic precursor of F_{420}) is used by *Streptomyces* species in tetracycline and lincomycin biosynthesis (Rhodes et al. 1981; McCormick and Morton 1982; Coats et al. 1989) and may be used in mitomycin C biosynthesis (Mao et al. 1999). In *Mycobacterium* and *Nocardia*, F_{420} is used by F_{420}-dependent glucose-6-phosphate dehydrogenase (Purwantini and Daniels 1996; Purwantini et al. 1997). The cyanobacteria *Synechococcus* sp. (previously known as *Anacystis nidulans*) and *Synechocystis* sp., and the green alga *Scenedesmus acutus* use F_{420} in their photolyase (Eker et al. 1988, 1990; Ng et al. 2000).

The structure of coenzyme F_{420} from *Methanobacterium thermoautotrophicum* (F_{420}–2), shown in Fig. 1A, contains a FO chromophore (Eirich et al. 1978, 1979). The side chain attached at the N-10 position contains ribityl, phosphate, and lactyl groups in sequence, terminating with two L-glutamate groups connected with a γ-glutamyl linkage. FO, a biosynthetic intermediate that lacks the phosphate-to-glutamate portion of the side chain, is found in cells and media of methanogens and actinomycetes (McCormick and Morton 1982; Kern et al. 1983; Kuo et al. 1989). *Methanosarcina barkeri* makes modified F_{420} structures thought to contain both four and five γ-linked glutamate groups (Gorris and van der Drift 1986; Peck 1989); however, no primary data have been published to establish the number of glutamate residues, and it has not been determined if they are linked by α- or γ-bonds. Precise structural work on *Streptomyces* 5-deazaflavins has been limited to FO (McCormick and Morton 1982; Kuo et al. 1989), but thin-layer electrophoresis of *Streptomyces griseus* F_{420} has suggested that more than two glutamate residues are present (Eker 1980). F_{420} from *Sulfolobus*, *Thermoplasma*, and halobacteria is reported to contain two or more glutamate groups, based on TLC (Lin and White 1986). F_{420} from *Mycobacterium avium* is reported to have one glutamate group, based on amino acid analysis (Naraoka et al. 1984).

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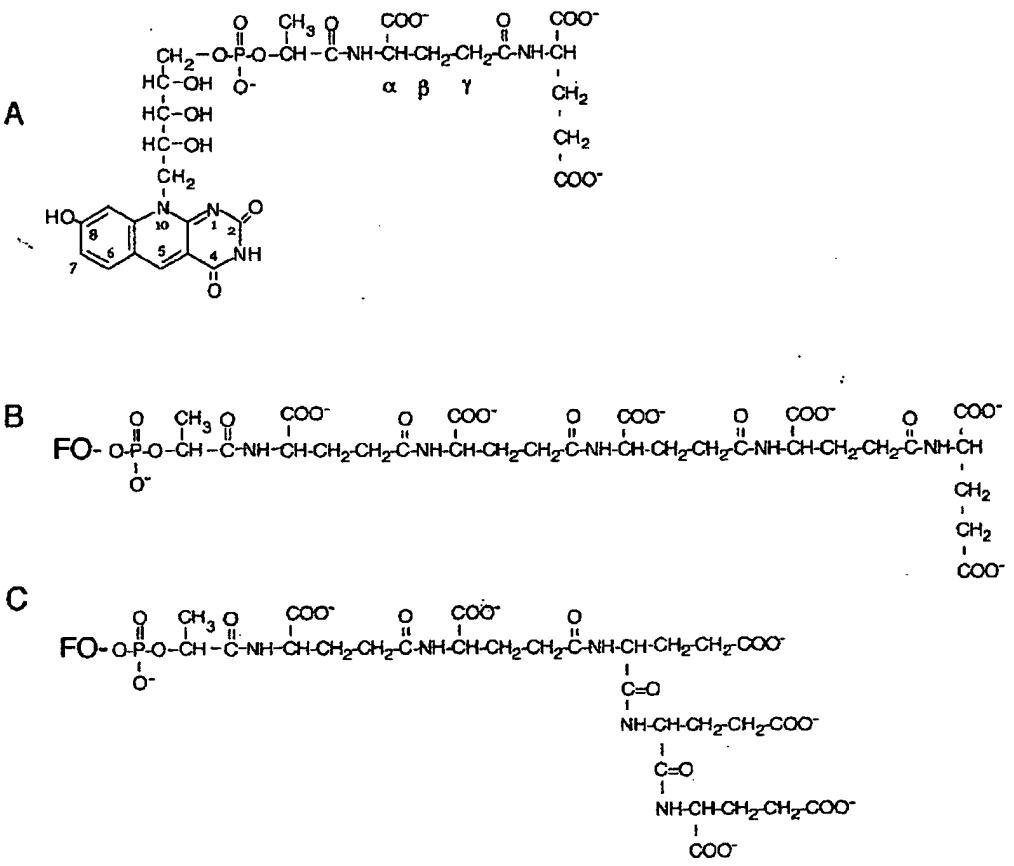

Fig. 1A–C Structure of F_{420}-2 from *Methanobacterium thermoautotrophicum*, and possible structures of the side chain in F_{420}-5 from *Mycobacterium* species. **A** *Methanobacterium thermoautotrophicum* F_{420}-2, with the second glutamate group linked by a γ-glutamyl bond. **B** Structure of pentaglutamate sidechain bound to the lactyl of F_{420} with the second to fifth glutamate residues linked by γ-glutamyl bonds, in analogy with eukaryotic folate; **C** Structure of pentaglutamate side chain with the second and third glutamate groups linked by γ-glutamyl bonds, but the fourth and fifth glutamate residues linked by α-glutamyl bonds, in analogy with *Escherichia coli* folate

The side chain of F_{420} is structurally analogous to the side chain of folic acid since glutamate groups are attached to the carboxyl of the lactyl moiety of the F_{420} instead of the carboxyl of the *p*-aminobenzoic moiety of folate. Both eukaryotes and prokaryotes contain folic acid with two to seven glutamate groups (Blakley and Benkovic 1984). All glutamate groups in eukaryotic folic acid are joined by a γ-glutamyl bond, whereas in *Escherichia coli* the second and third glutamate groups are linked by γ-glutamyl linkages, and the remaining glutamate groups are linked by α-glutamyl bonds (Ferone et al. 1986; Johnson et al. 1988). Sarcinopterin, an analogue of folic acid in *Methanosarcina barkeri*, contains a glutamate attached via an amide bond to the α-carboxylic acid group of the hydroxyglutaric moiety of methanopterin (van Beelen et al. 1984), which establishes precedence for an α-glutaryl linkage in an organism that makes F_{420}.

Our initial HPLC analysis of *Mycobacterium smegmatis* suggested that several polyglutamate F_{420} species were present. The glutamate groups could have been linked by either α- or γ-glutamyl linkages. As depicted in Fig. 1B, a pentaglutamate F_{420} could be analogous to eukaryotic folate (with all glutamate groups linked with the γ-carboxyl of the prior glutamate). Alternatively, as in Fig. 1C, F_{420} could be analogous to *E. coli* folate (with the terminal two glutamate groups linked to the α-carboxyl of the prior glutamate), or any mixture of glutamyl bonds may have been present. We report here that the predominant F_{420} in all *Mycobacterium* species examined contain four to seven glutamate molecules, unlike the monoglutamate structure proposed earlier for *M. avium* F_{420} (Naraoka et al. 1984), and we also provide evidence that all the glutamate molecules are the L-isomer, and are all linked by γ-glutamyl bonds.

Materials and methods

Bacterial strains, media, and growth conditions

Mycobacterium smegmatis mc²155 was a gift of Dr. W. Jacobs Jr. (Howard Hughes Medical Institute, Albert Einstein College of Medicine, New York). *M. avium* ATCC 25291, *Mycobacterium bovis* BCG (Montreal) ATCC 35746, and *Mycobacterium fortuitum* ATCC 6841 were obtained from the ATCC (Manassas, Va.). The above mycobacteria were typically grown in Middlebrook 7H9 medium (Difco) supplemented with 0.2% glycerol and 0.05% Tween 80, except that Bacto Middlebrook ADC Enrichment was added for growth of *M. bovis* and *M. avium*. To test the role of glutamate in the medium on F_{430} structure, *M. smegmatis* was grown in two other media. One contained a high level of glutamate (g per liter): K_2HPO_4 (0.5), $MgSO_4$ (1), sodium citrate (2), ferric ammonium citrate (0.05), sodium glutamate (40), Tween 80 (0.5), and glycerol (60) (Naraoka et al. 1984). Another medium with no glutamate contained (g per liter): NaH_2PO_4 (3.44), KH_2PO_4 (4.0), $MgSO_4 \cdot 7H_2O$ (2.5), sodium citrate·$2H_2O$ (2.5), ferrous ammonium sulfate (0.01), L-asparagine (5.0), glycerol (25) (Bowles and Segal 1965). *M. tuberculosis* strains $H_{37}Rv$ and CSU93 were grown at Colorado State University by Dr. John Belisle using glycerol-alanine-salts medium in flasks with gentle agitation for about 14 days. *Methanobacterium thermoautotrophicum* strain Marburg was grown in a 100-l fermentor on H_2:CO_2 (80:20, v/v), and *Methanosarcina barkeri* was grown in a carboy on methanol as described previously (Daniels 1995).

A richer medium was used to grow *M. smegmatis* for large-scale preparation of F_{420} for NMR analysis. This medium contained (g per liter): glucose (15), blackstrap molasses (20), soluble starch (40), cottonseed hydrolysate (25), $CaCO_3$ (8), and K_2SO_4 (2) (Kuo et al. 1989). After 8–9 days of growth (750 ml in 2.8-l Fernbach flasks), the cells were harvested, washed in distilled water and the pellet was frozen until use.

HPLC methods

Different forms of F_{420} were separated by HPLC using a Beckman System Gold 126 HPLC with a Shimadzu RF-10Axl fluorescence detector (excitation at 400 nm, emission at 470 nm). Analytical HPLC was carried out using a method similar to that of Gorris and van der Drift (1988), with modifications. A 3.9×300 mm α-Bondpack C-18 (Supleco) column was eluted at 1 ml/ min. Buffer A was 27.5 mM sodium acetate (pH 4.7) containing 2% acetonitrile; buffer B was 100% acetonitrile. The portion of buffer B in the elution buffer was varied as follows: 0–2 min, 0%; 2–6 min, 0–2%; 6–15 min, 2–10%; 15–22 min, 28%; 22–27 min, 28–0% (all linear gradients). F_{420} prepared for NMR spectroscopy was further purified by two semi-preparative HPLC methods with a 10×250 mm Supelcosil LC-18 column eluted at 3 ml/min. The first method was identical to the analytical method except for flow rate and column. The second was isocratic methanol:water (20:80, v/v). The product of F_{420} degradation by carboxypeptidase G was purified by HPLC using no sodium acetate for the acetonitrile elution.

Purification of F_{420} and FO

Mycobacterium smegmatis from 3 l of molasses medium culture was extracted by boiling in 150 ml of 50 mM sodium phosphate buffer (pH 7) for 30 min. After centrifugation and removal of the supernatant, the pellet was reextracted with 150 ml buffer and the supernatants were combined and filtered with a 0.45-μm filter. The extract was applied in three separate loadings of ~110 ml each to a QAE-Sephadex A-25 column (5×12 cm) equilibrated with 50 mM sodium phosphate (pH 7). FO and F_{420} were eluted with a 0–1 M NaCl gradient (total volume, 1,400 ml) in the samebuffer using a Biorad chromatography system and a fluorescence detector (Gilson Model 121, Middleton, Wis.). FO and F_{420} gave two well-resolved fluorescent peaks that were collected separately. These separate peaks were adjusted to pH 4.7 and loaded onto a 1.5×7 cm column packed with Sephasil C18 (Pharamacia Biotech) that had been equilibrated with 25 mM sodium acetate, pH 4.7. FO and F_{420} were eluted with a 0–80% methanol gradient in the same buffer (total volume, 280 ml). Peaks from this column were adjusted to pH 7 and run on a second QAE column (1.5×15 cm). The amount of F_{420} (4.05 mg) and FO (1.7 mg) from the 3 l of culture were estimated by A_{420} (ε_{420}=38.5 mM^{-1} cm^{-1}, pH 7.0). A portion of this F_{420} was then chromatographed by semi-preparative HPLC using both the acetonitrile and methanol-water techniques described in the HPLC methods. F_{420} from *Methanobacterium thermoautotrophicum* was purified with the same procedures.

NMR and amino acid analysis

^{1}H-NMR spectra were obtained on a Varian Inova 500 MHz spectrometer in the University of Iowa College of Medicine NMR facility. Up to 2,048 transients were averaged using a 6-s relaxation-delay, a 6.9-μs (90°) pulse width and a 2.73-s acquisition time. The spectral width was 24 ppm, centered on the HDO peak at 4.757 ppm. The HDO signal was suppressed by continuous wave presaturation during the recycle delay; 32,768 complex points were collected, zero filled to 128 K and Fourier-transformed using a 0.5-Hz Lorenzian line broadening. Spectra were baseline-corrected using a spline function before peak integrals were measured. Samples were analyzed in Wilmad 535 tubes using Doty susceptibility-matched volume-reducing plugs (Wilmad Glass, Buena, N.J.).

F_{420} and the product of carboxypeptidase G F_{420} degradation were hydrolyzed at 110°C in 6 N HCl for 24 h, then analyzed by the UI Molecular Analysis Facility for amino acid content by HPLC with a cation exchange column and conventional automated ninhydrin analysis.

Peptidase reactions

Mycobacterium smegmatis F_{420} (6.5 μg) was incubated with peptidases to determine if the glutamate chains were degraded. The carboxypeptidase Y reaction was carried out by mixing F_{420} with 100 μl of 25 mM sodium citrate (pH 5.5) and 4–6 units of carboxypeptidase Y (Sigma or ICN Biomedicals, both from bakers yeast). The carboxypeptidase G reaction was carried out by mixing F_{420} with 100 μl of 25 mM Tris-HCl (pH 7.3) and 1.25–4 units of carboxypeptidase G (Sigma, from *Pseudomonas*). The γ-glutamyltranspeptidase reaction was carried out by mixing F_{420} with 50 μl of 25 mM ammonium acetate (pH 8.8) and 50 μl of γ-glutamyltranspeptidase (4–8 units; equine or bovine kidney, Sigma) dissolved in 0.1 M Tris-10 mM $MgCl_2$ (pH 9.0.). The reactions were started by the addition of F_{420}, and incubated at 30°C. Samples were taken at various times between 0 and 50 h, boiled, centrifuged to remove precipitated protein, and frozen until analyzed by HPLC. Reactions with boiled enzymes were used as controls.

Results and discussion

NMR analysis of F_{420}

Figure 2 shows the proton NMR spectrum of F_{420} from *M. smegmatis*. This F_{420} was the major peak purified with preparative HPLC, and showed two partially overlapping peaks when examined by analytical HPLC. Table 1 provides a quantitative comparison of these F_{420} NMR data with data collected with F_{420}-2 from *Methanobacterium thermoautotrophicum*. Overall, our NMR data on F_{420} and FO are in agreement with the data of Eirich et al. (1978)

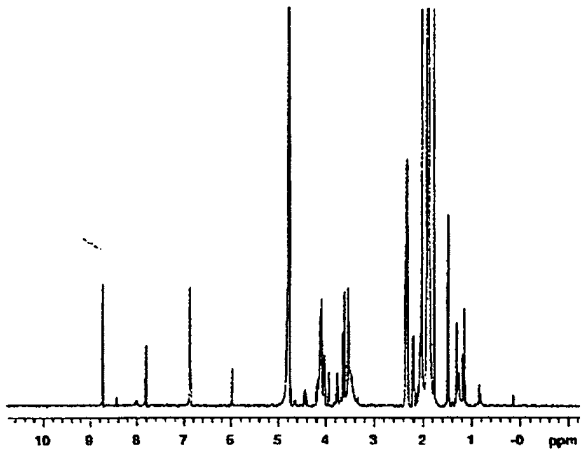

Fig. 2 Proton NMR spectrum of *Mycobacterium smegmatis* F_{420}

and Kuo et al. (1989), and we have used their assignments as a guide for our work. The aromatic protons in positions C-5, C-6, C-7, and C-9 were approximately one each in F_{420} from both sources, suggesting that the chromophore is identical in both organisms. This was in agreement with our proton NMR analysis of *M. smegmatis* FO, and with UV-visible spectra for F_{420} from mycobacteria and methanogens (data not shown). The signal at 1.49 ppm yielded 2.78 protons for *M. smegmatis* F_{420}, which matched well with the corresponding methanogen F_{420} signal of 3.07 protons, indicating the presence of one lactyl methyl group. An accurate measure of the number of glutamate molecules present was obtained with the 10.06 protons observed at 2.34 ppm (from the glutamate γ-methylene group, indicated in Fig. 1A), which corresponds to 5.03 glutamate molecules; a repeat of this NMR analysis resulted in an average of 5.3 glutamate molecules. NMR of this region with the methanogen F_{420} revealed 4.17 protons, consistent with the two glutamate molecules expected. Analysis of the *M. smegmatis* F_{420} region of 3.8–5.1 ppm, which should represent a mixture of ribityl group protons and glutamate methine protons, was complicated by possible errors in subtracting the water peak from this region, but was consistent with the presence of the seven non-hydroxyl ribityl group protons and four glutamate molecules. The strong signal at 1.85, and its associated sidebands, arise from the methyl group of acetate, which remained as a contaminant after its use in HPLC purification of F_{420}.

Amino acid analysis of F_{420}

Amino acid analysis of F_{420} indicated that 5.20 glutamate molecules were present per molecule of *M. smegmatis* F_{420}. Small amounts of glycine (less than one molecule per F_{420} molecule) were generally seen, and smaller amounts of lysine were occasionally seen. Both are likely due to trace contaminants from sample processing. These data, coupled with NMR analysis (above) and HPLC analysis (below), indicate that the two species in the sample analyzed are principally F_{420}–5 and F_{420}–6, averaging to "F_{420}–5.3."

Table 1 Proton NMR data for F_{420} purified from *Mycobacterium smegmatis* and *Methanobacterium thermoautotrophicum*. *(s)* Singlet, *(d)* doublet, *(m)* multiplet

F_{420} source	Carbon atom	Chemical shift in ppm	Protons[a]	
			Observed	Expected
Mycobacterium smegmatis F_{420}–5.3[b]	5	8.74 (s)	0.94	1.0
	6	7.82 (d)	1.00	1.0
	7+9	6.89 (m)	2.02	2.0
	1'-5'+Glu methine	3.8–5.1 (m)[c]	10.56	12.3
	Glu γ-methylene	2.34 (m)	10.06	10.6
	Lactyl methyl	1.49 (d)	2.78	3.0
Methanobacterium thermoautotrophicum F_{420}–2	5	8.68 (s)	1.03	1.0
	6	7.79 (d)	1.00	1.0
	7+9	6.85 (m)	2.02	2.0
	1'-5'+Glu methine	3.7–5.1 (m)[c]	6.45	9.0
	Glu γ-methylene	2.35 (m)	4.17	4.0
	Lactyl methyl	1.49 (d)	3.07	3.0

[a]The number of protons in the integration, normalized with C-6 equal to one

[b]The F_{420} used for NMR was analyzed by HPLC and found to contain principally what are thought to be F_{420} with 5- or 6-glutamate residues. The calculated average corresponds to 5.3 glutamate residues

[c]This region includes seven non-OH protons from the ribityl molecule and one methine proton from each glutamate residue; the water proton peak at 4.85 ppm was subtracted, leading to more potential error than with other assignments

HPLC analysis of F_{420} from mycobacteria and methanogens

Figure 3 provides examples of HPLC profiles of samples extracted from three microorganisms. C-18 reverse-phase HPLC has been used previously to distinguish F_{420} species hypothesized to have varying numbers of glutamate residues; the more glutamate residues, the more charge and the shorter the retention time (Gorris and van der Drift 1986, 1988; Peck 1989). The deazaflavin with the least charge, FO, binds most tightly. *Methanobacterium thermoautotrophicum* extract contained principally F_{420}–2, consistent with structural NMR data above, and published information (Eirich et al. 1978), as well as a moderate amount of FO and a small amount of what we believe is F_{420}–3. *Methanosarcina barkeri* extract contained mostly a mixture of F_{420}–4 and F_{420}–5, in agreement with its anticipated composition(Gorris and van der Drift 1986; Peck 1989). *M. smegmatis* extract contained mostly a mixture of F_{420}–5 and F_{420}–6, in agreement with our NMR data above, as well as a small amount of what we believe to be F_{420}–7.

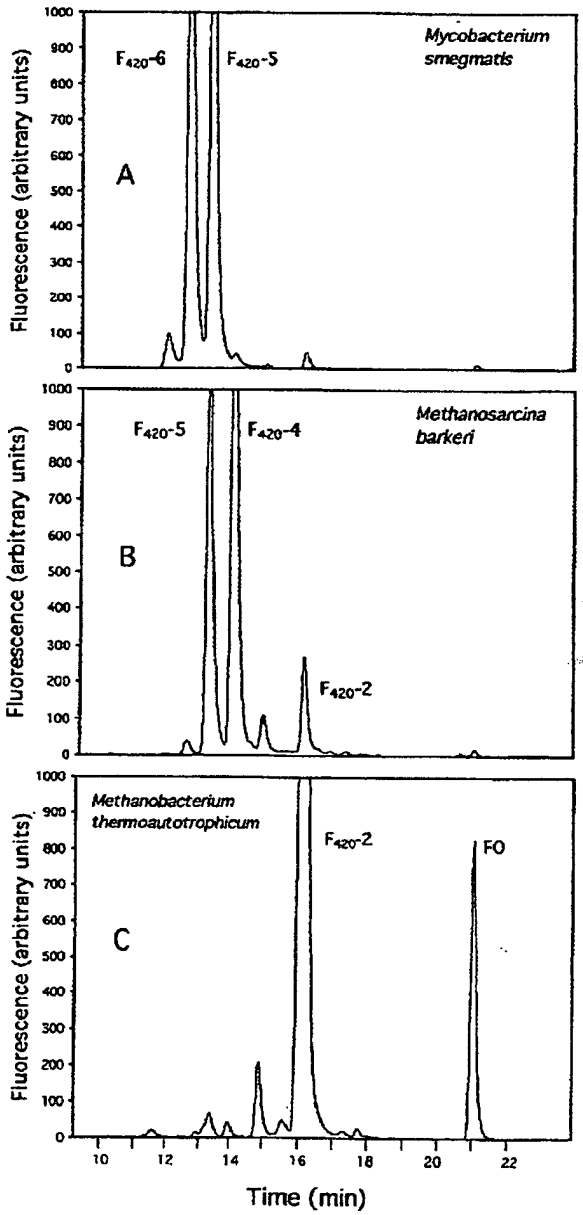

Fig. 3 HPLC elution profiles of extract prepared from **A** *Mycobacterium smegmatis*, **B** *Methanosarcina barkeri*, and **C** *Methanobacterium thermoautotrophicum*

Table 2 provides quantitative HPLC data on these organisms and a range of other mycobacteria. Of particular interest, all *Mycobacterium* species, including *M. avium*, have major amounts of only F_{420}–4 to F_{420}–7; five andsix glutamate residue forms generally predominate. In the mycobacteria, FO accounted for about 1–7% of the total deazaflavin. There is no evidence for significant levels of F_{420}–1 in any *Mycobacterium* species. This is in contrast to a previous report that F_{420}–1 was the sole F_{420} species in *M. avium* (Naraoka et al. 1984). Due to this conflict, we examined *M. smegmatis* grown in the medium with high glutamate levels used by Naraoka et al. (1984) to see if F_{420}–1 was predominant or detectable. As shown in Table 2, this was not the case. If glutamate-free medium was used, the major structures were still F_{420}–5 and F_{420}–6 (data not shown). We believe that the incorrect assignment of the F_{420} structure in *M. avium* by Naraoka et al. et al. (1984) was due to acid hydrolysis that removed most glutamate groups, as a result of the use of HCl as a chromatographic eluant, particularly if a fraction was concentrated by rotary evaporation.

Degradation of F_{420} by peptidases

Several peptidases have been used by others to evaluate glutamate linkages in folic acid and F_{420}. Carboxypeptidase Y is reported to cleave only α-glutamyl bonds (bonds between the NH-group of a glutamate and the α-carboxyl of the previous glutamate), and has been used with *E. coli* folic acid (Ferone et al. 1986; Johnson et al. 1988). Incubation of F_{420} with carboxypeptidase Y from two different suppliers failed to show F_{420} degradation. This suggests that no α-glutamyl bonds are present, but it is possible that F_{420}, as opposed to folic acid, is a poor substrate.

Commercially available crude preparations of γ-glutamyltranspeptidase have been used to cleave glutamyl-glutamyl bonds in F_{420} (Lin and White 1986) and in folates (Blakley and Benkovic 1984). We also found that glutamate residues were slowly cleaved from *M. smegmatis* F_{420} by these preparations, to yield (after 24–48 h) three small F_{420} derivatives that are not FO, but which may be F_{420}–1, FO-phosphate, and F_{420}–0 (FO-phosphate-lactyl), based on their elution between F_{420}–2 and FO (datanot shown). Unfortunately, purified enzymes of this

Table 2 Relative amounts of F_{420} types and FO in several *Mycobacterium* species and in two methanogen species. The total HPLC peak area of the F_{420} species, plus FO, add up to 100%. The numbers 7 to 2 refer to the numbers of glutamate molecules on F_{420}, corresponding to F_{420}–7 to F_{420}–2. Very small peaks that may represent F_{420}–1 and F_{420}–8 are not included

Organism	Relative amounts of F_{420} species and FO in cells (percent of total F_{420}+FO)						
	7	6	5	4	3	2	FO
Methanosarcina barkeri	0.1	1.5	39.4	49.2	3.5	5.7	0.6
Methanobacterium thermoautotrophicum	0.38	0.12	0.55	0.75	3.7	80.3	14.4
Mycobacterium smegmatis	3.9	45.8	46.8	2.0	0.4	0.8	0.4
Mycobacterium smegmatis (high Glu)	3.5	33.6	51.5	7.4	2.2	0.2	1.7
Mycobacterium avium	28.0	40.4	24.0	0.0	0.4	0.3	6.9
Mycobacterium bovis BCG	9.6	36.2	35.4	10.8	2.6	2.8	2.6
Mycobacterium tuberculosis H37Rv	3.1	36.5	42.8	8.7	3.8	1.9	3.3
Mycobacterium tuberculosis CSU93	2.9	34.7	39.1	11.1	5.8	2.3	4.2
Mycobacterium fortuitum	0.4	14.4	62.8	15.4	3.7	1.5	1.8

type are not available from commercial suppliers, and since these peptidases were crude preparations, other activities could have been present, reducing the certainty that only γ-linked glutamate groups were present. As an alternative, we obtained a partly purified γ-glutamyl hydrolase (carboxypeptidase G) from *Pseudomonas* species that is reported to remove glutamate from methotrexate and folic acid, and to cleave peptides containing L-glutamate and not D-glutamate (Goldman and Levy 1967; Levy and Goldman 1967). This enzyme removed glutamate groups from F_{420}–5 and F_{420}–6 to form only one HPLC product consistent with an F_{420} derivative with one or no glutamate molecule (based on its retention time between F_{420}–2 and FO). We purified 16 nmol of this product, and determined by amino acid analysis that it contained no glutamate residue. Although its precise structure is unknown, this product is in agreement with the removal of the sole glutamate molecule from methotrexate and folic acid by carboxypeptidase G to yield a free carboxyl group and free glutamate, as observed previously (Goldman and Levy 1967; Levy and Goldman 1967). We speculate that F_{420} is converted by carboxypeptidase G to F_{420}–0, which contains the phosphate and lactyl groups, but no glutamate. Thus, our data showing that glutamate residues on F_{420} are not removed by carboxypeptidase Y, but are fully removed by carboxypeptidase G, are consistent with a γ-linked L-glutamate chain on *Mycobacterium* F_{420}, as depicted in Fig. 1B. Also of importance to our work is that the small F_{420} derivatives produced by γ-glutamyltranspeptidase degradation, and the putative F_{420}–0 produced by carboxypeptidase G, may be valuable molecules for the assay of enzymes of the F_{420} biosynthesis pathway that add glutamate or lactyl groups.

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References

Blakley RL, Benkovic SJ (1984) Folates and pterins: Chemistry and biochemistry of folates. Wiley, New York

Bowles JA, Segal W (1965) Kinetics of utilization of organic compounds in the growth of *Mycobacterium tuberculosis*. J Bacteriol 90:157–163

Coats JH, Li GP, Kuo MS, Yurek DA (1989) Discovery, production, and biological assay of an unusual flavenoid cofactor involved in lincomycin biosynthesis. J Antibiot (Tokyo) 42:472–474

Daniels L (1994) The biochemistry of methanogenesis. In: Kates M, Kushner DJ, Matheson AT (eds) The biochemistry of archaea. Elsevier, Amsterdam

Daniels L (1995) Large scale culturing of methanogenic bacteria. In: Robb FT, Place AR, Sowers KR, Schreier HJ, DasSarma S, Fleischmann EM (eds) Archaea: a laboratory manual. Cold Spring Harbor, Cold Spring Harbor, New York, pp 63–74

DiMarco AA, Bobik TA, Wolfe RS (1990) Unusual coenzymes of methanogenesis. Annu Rev Biochem 59:355–394

Eirich LD, Vogels GD, Wolfe RS (1978) Proposed structure for coenzyme F_{420} from *Methanobacterium*. Biochemistry 17:4583–4593

Eirich LD, Vogels GD, Wolfe RS (1979) Distribution of coenzyme F_{420} and properties of its hydrolytic fragments. J Bacteriol 140:20–27

Eker AP (1980) Photoreactivating enzyme from *Streptomyces griseus*–II. Evidence for the presence of an intrinsic chromophore. Photochem Photobiol 32:593–600

Eker AP, Hessels JKC, van de Velde J (1988) Photoreactivating enzyme from the green alga *Scenedesmus acutus*. Evidence for the presence of two different flavin chromophores. Biochemistry 27:1758–1765

Eker AP, Kooiman P, Hessels JK, Yasui A (1990) DNA photoreactivating enzyme from the cyanobacterium *Anacystis nidulans*. J Biol Chem 265:8009–8015

Ferone R, Hanlon MH, Singer SC, Hunt DF (1986) α-Carboxyl-linked glutamates in the folylpolyglutamates of *Escherichia coli*. J Biol Chem 261:16356–16362

Goldman P, Levy CC (1967) Carboxypeptidase G: purification and properties. Proc Natl Acad Sci USA 58:1299–1306

Gorris LGM, van der Drift C (1986) Methanogenic cofactors in pure cultures of methanogens in relation to substrate utilization. In: Dubourguier HC, Albagnac G, Montreuil J, Romond C, Sautiere P, Guillaume J (eds) Biology of anaerobic bacteria. Elseivier, Amsterdam, pp 144–150

Gorris LGM, van der Drift C (1988) Separation and quantitation of cofactors from methanogenic bacteria by high-performance liquid chromatography: optimum and routine analysis. J Microbiol Methods 8:175–190

Hartzell PL, Zvilius G, Escalante-Semerena JC, Donnelly MI (1985) Coenzyme F_{420} dependence of the methylenetetrahydromethanopterin dehydrogenase of *Methanobacterium thermoautotrophicum*. Biochem Biophys Res Commun 133:884–890

Jacobson FS, Daniels L, Fox JA, Walsh CT, Orme-Johnson WH (1982) Purification and properties of an 8-hydroxy-5-deazaflavin-reducing hydrogenase from *Methanobacterium thermoautotrophicum*. J Biol Chem 257:3385–3388

Johnson JL, Hamm-Alvarez S, Payne G, Sancar GB, Rajagopalan KV, Sancar A (1988) Identification of the second chromophore of *Escherichia coli* and yeast DNA photolyases as 5,10-methenyltetrahydrofolate. Proc Natl Acad Sci USA 85:2046–2050

Jones JB, Stadtman TC (1980) Reconstitution of a formate-NADP+ oxidoreductase from formate dehydrogenase and a 5-deazaflavin-linked NADP+ reductase isolated from *Methanococcus vannielii*. J Biol Chem 255:1049–1053

Kern R, Keller PJ, Schmidt G, Bacher A (1983) Isolation and structural identification of a chromophoric coenzyme F_{420} fragment from culture fluid of *Methanobacterium thermoautotrophicum*. Arch Microbiol 136:191–193

Kunow J, Linder D, Stetter KO, Thauer RK (1994) $F_{420}H_2$: quinone oxidoreductase from *Archaeoglobus fulgidus*. Characterization of a membrane-bound multisubunit complex containing FAD and iron-sulfur clusters. Eur J Biochem 223:503–511

Kuo MS, Yurek DA, Coats JH, Li GP (1989) Isolation and identification of 7,8-didemethyl-8-hydroxy-5- deazariboflavin, an unusual cosynthetic factor in streptomycetes, from *Streptomyces lincolnensis*. J Antibiot (Tokyo) 42:475–478

Levy CC, Goldman P (1967) The enzymatic hydrolysis of methotrexate and folic acid. J Biol Chem 242:2933–2938

Lin XL, White RH (1986) Occurrence of coenzyme F_{420} and its gamma-monoglutamyl derivative in nonmethanogenic archaebacteria. J Bacteriol 168:444–448

Ma K, Thauer RK (1990) Purification and properties of N5, N10-methylene-tetrahydromethanopterin reductase from *Methanobacterium thermoautotrophicum* (strain Marburg). Eur J Biochem 191:187–193

Mao Y, Varoglu M, Sherman DH (1999) Molecular characterization and analysis of the biosynthetic gene cluster for the antitumor antibiotic mitomycin C from *Streptomyces lavendulae* NRRL 2564. Chem Biol 6:251–263

McCormick JRD, Morton GO (1982) Identity of cosynthetic Factor 1 of *Streptomyces aureofaciens* and fragment FO from coenzyme F_{420} of *Methanobacterium* sp. J Amer Chem Soc 104: 4014–4015

Naraoka T, Mamoi K, Fukasawa K, Goto M (1984) Isolation and identification of a naturally occuring 7,8-didemethyl-8-hydroxy-5-deazaflavin derivative from *Mycobacterium avium*. Biochim Biophys Acta 797:377–380

Ng WO, Zentella R, Wang Y, Taylor J-SA, Pakrasi HB (2000) *phrA*, the major photoreactivationg factor in the cyanobacterium *Synechocystis* sp. strain PCC 6803 codes for a cyclobutane-pryimidine-dimer-specific DNA photolyase. Arch Microbiol 173:412–417

Peck MW (1989) Changes in concentrations of coenzyme F_{420} analogs during batch growth of *Methanosarcina barkeri* and *Methanosarcina mazei*. Appl Environ Microbiol 55:940–945

Purwantini E, Daniels L (1996) Purification of a novel coenzyme F_{420}-dependent glucose-6-phosphate dehydrogenase from *Mycobacterium smegmatis*.J Bacteriol 178:2861–2866

Purwantini E, Gillis T, Daniels L (1997) Presence of F_{420}-dependent glucose-6-phosphate dehydrogenase in *Mycobacterium* and *Nocardia* species, but absence in *Streptomyces* and *Corynebacterium* species and methanogenic Archaea. FEMS Microbiol Lett 146:129–134

Rhodes PM, Winskill N, Friend EJ, Warren M (1981) Biochemical and genetic comparison of *Streptomyces rimosus* mutants impaired in oxytetracycline biosynthesis. J Gen Microbiol 124: 329–338

Van Beelen P, Labro JF, Keltjens JT, Geerts WJ, Vogels GD, Laarhoven WH, Guijt W, Haasnoot CA (1984) Derivatives of methanopterin, a coenzyme involved in methanogenesis. Eur J Biochem 139:359–365

Widdel F, Wolfe RS (1989) Expression of secondary alcohol dehydrogenase in methanogenic bacteria and purification of the F_{420}-specific enzyme from *Methanogenium thermophilum* strain TCI. Arch Microbiol 152:322–328